Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/EP04/014772

International filing date: 22 December 2004 (22.12.2004)

Document type: Certified copy of priority document

Document details: Country/Office: EP
                     Number: 04075249.5
                     Filing date: 26 January 2004 (26.01.2004)

Date of receipt at the International Bureau: 08 February 2005 (08.02.2005)

Remark: Priority document submitted or transmitted to the International Bureau in
           compliance with Rule 17.1(a) or (b)

PATENT COOPERATION TREATY

From the RECEIVING OFFICE

To:

☒ The International Bureau of WIPO
34, chemin des Colombettes
1211, Geneva 20
Suisse

☐ The International Searching Authority

PCT

NOTIFICATION CONCERNING DOCUMENTS TRANSMITTED

Date of mailing (day/month/year)	07. 02. 2005

International application No.

PCT/EP2004/014772

The receiving Office transmits herewith the following documents:

REC'D 0 6 FEB 2005
WIPO   PCT

1. ☐ the record copy (Article 12(1)) (only for the IB).

2. ☐ the search copy of form PCT/RO/101 (Article 12(1)) (only for the ISA).

3. ☐ the confirmation copy (Administrative Instructions, Section 331) (only for the IB).

4. ☐ **substitute** sheets (Administrative Instructions, Section 325(a)).

5. ☐ **later** submitted sheets (Administrative Instructions, Section 309(b)(iii), (c)(ii)).

6. ☐ **later** submitted drawings (Administrative Instructions, Section 310(c)(iii), (d)(ii)).

7. other document(s):
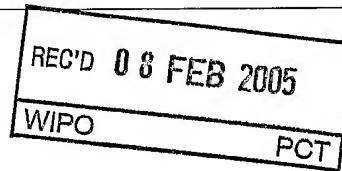
    ☐ letter(s) dated: _____ .

    ☐ _____ power(s) of attorney (only for the IB).

    ☐ statement(s) explaining lack of signature considered to be satisfactory by this receiving Office (only for the IB).

    ☒ 1 priority document(s) (only for the IB).

    ☐ fee calculation sheet (only for the IB).

    ☐ document(s) concerning deposited biological material.

    ☐ nucleotide and/or amino acid sequence listing(s) in computer readable form (only for the ISA).

    ☐ PCT EASY diskette (only for the IB).

    ☐ earlier search(es) (only for the ISA).

    ☐ Form PCT/RO/106.

    ☐ Form PCT/RO/_____ .

    ☐ _____ .

    ☐ _____ .

Name and mailing address of the Receiving Office	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040 Fax: (+31-70) 340-3016	J.C. de BRUIJN

Form PCT/RO/118 (January 2003; reprint January 2004)

PCT/EP200 4 / 0 1 4 7 7 2

Europäisches
Patentamt

European
Patent Office

Office européen
des brevets

Bescheinigung   Certificate   Attestation

Die angehefteten Unterla-
gen stimmen mit der
ursprünglich eingereichten
Fassung der auf dem näch-
sten Blatt bezeichneten
europäischen Patentanmel-
dung überein.

The attached documents
are exact copies of the
European patent application
described on the following
page, as originally filed.

Les documents fixés à
cette attestation sont
conformes à la version
initialement déposée de
la demande de brevet
européen spécifiée à la
page suivante.

Patentanmeldung Nr.   **Patent application No.**   **Demande de brevet n°**

04075249.5

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

R C van Dijk

Europäisches Patentamt **European Patent Office** **Office européen des brevets**

Anmeldung Nr:
Application no.:   04075249.5
Demande no:

Anmeldetag:
Date of filing: 26.01.04
Date de dépôt:

Anmelder/Applicant(s)/Demandeur(s):

UNILEVER N.V.
Weena 455
3013 AL Rotterdam
PAYS-BAS

Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se referer à la description.)

Enzymatic modification of triglyceride fats

In Anspruch genommene Priorität(en) / Priority(ies) claimed /Priorité(s) revendiquée(s)
Staat/Tag/Aktenzeichen/State/Date/File no./Pays/Date/Numéro de dépôt:

Internationale Patentklassifikation/International Patent Classification/
Classification internationale des brevets:

A23D9/00
A23D7/00

Am Anmeldetag benannte Vertragstaaten/Contracting states designated at date of
filing/Etats contractants désignées lors du dépôt:

AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LU MC NL
PT RO SE SI SK TR LI

5

Enzymatic modification of triglyceride fats

10 **Field of the invention**

The present invention deals with an interesterification
process of triglyceride fats. More particularly the process
concerns an enzymatic interesterification process which is
15 further denoted as an enzymatic rearrangement process.

Chemical interesterification of a triglyceride fat aims at
an exchange of the fatty acid residues connected to the
glyceride moiety of the fat. In the resulting triglycerides
20 fatty acid residues have been exchanged by other residues,
originating from the same or different glyceride molecules
or from free fatty acids when present in the reaction
mixture.

25 Such chemical interesterification process needs a catalyst,
which usually is an alkali metal hydroxide or an alkali
metal alkanolate, such as sodium methanolate. The exchange
of fatty acid residues eventually results in a statistically
random distribution of the fatty acid residues over the
30 terminal and middle positions of the glyceride molecule. The
obtained fat is said to have become fully randomised.

2

Since consumers increasingly prefer food and food
ingredients which have not been exposed to a chemical
treatment, a general need has arisen for non-chemical
modification processes of triglyceride fats. Chemical
5 interesterification may be substituted by an enzymatic
rearrangement process. Such process does not affect the
naturalness of the starting fat.

For enzymatic rearrangement (ER) a lipase enzyme is used as
10 catalyst. Lipases used for ER comprise the microbial Mucor
miehei lipase and Thermomyces lanuginosa lipase. These
lipases are 1,3-specific which means that they only affect
fatty acid residues on the terminal positions of the
glyceride moiety of the fat molecule. The ester bond on the
15 middle position remains unaffected, with the result that the
rearrangement process is limited to the terminal positions.
Thermomyces lanuginosa lipase immobilized as an aggregate
with silica is a food grade catalyst and available on
industrial scale under the name Lipozyme TL IM™.
20

WO96/14756 describes ER of fat blends using 1,3-specific
SP392 as lipase catalyst. The process is characterized in
that the rearrangement does not proceed beyond a conversion
degree of 90% (but being at least 20%).
25

An ER process which affects only the terminal fatty acid
residues delivers triglyceride products with a triglyceride
composition which is quite different from the fully
randomised triglyceride fat resulting from chemical
30 interesterification. A first adverse consequence is that the
extensive knowledge and experience built for the use of
fully randomised chemically interesterified fats in the
manufacture of food products can not be used. Another

adverse consequence is that 1,3-specific ER is not able to
increase the amount of triglycerides with a saturated middle
position which position usually is unsaturated in a natural
feedstock fat.

5

Attempts have been made to modify the ER process such that
also the middle position is randomized.
Some rare lipases including *Candida cylindracae* and
Arthrobacter lipases are non-specific. An ER process using
10 those lipases delivers a fully randomised fat, a fat
randomised on all glyceride positions. However, those
lipases either have been found to be not suited for
upscaling to an industrial modification process and/or have
not been approved for food manufacture.
15

The process described in EP 652289 uses a common 1,3-
specific lipase and a substantial amount, at least 4 wt.% of
diacylglycerides (also denoted as diglycerides). The
resulting fat becomes fully randomised over all positions,
20 but still contains the starting high amount of diglycerides
and other byproducts which need to be removed by a
subsequent purification process.

Recently, processes for enzymatic rearrangement have been
25 reported where the above mentioned Lipozyme TL IM catalyst
is employed: JAOCS, Vol.78, no 1 (2001) pp. 57-64 and JAOCS,
Vol.79, no 6 (2002) pp. 561-565. In order to obtain 100%
rearrangement relatively long enzyme contact times are
needed. The corresponding low reactor throughput makes the
30 process relatively expensive. Use of the process for
randomising the fatty acid residues over all glyceride
positions has not been considered and even is undesired
because the process aims at selective 1,3-rearrangement.

A cost effective ER process which delivers a fat with a highly randomised middle position is badly needed.

5

STATEMENT OF THE INVENTION

A process has been found comprising the exposure of a triglyceride fat to a catalyst comprising a lipase resulting

10 into a rearrangement of the fatty acid residues on the glyceride moiety over the terminal and middle positions, which rearrangement proceeds to a degree of conversion Re, where Re is selected from the range 0.3-0.95, the rearrangement comprising a randomisation of the fatty acid

15 residues on the middle position to a degree of conversion Ra, where Ra is selected from the range 0.06-0.75, which process is characterized by the use of a Thermomyces lanuginosa lipase containing catalyst having an activity which is at least 250 IUN, while $Ra > 0.32Re - 0.08$, the

20 catalyst activity and Ra and Re being as defined in the specification.

DETAILS OF THE INVENTION

25

The present process uses the Thermomyces lanuginosa lipase containing ER catalyst Lipozyme TL IM™ which is commercially available from NOVOZYMES, Denmark as an aggregate of enzyme and silica and having varying activity values, including

30 activities being at least 250 IUN. The method for establishing catalyst activity is described below. The invented process has the benefit over the prior art process that it is a one step process which does not need

undesirably high amounts of diglycerides for randomising the middle position of the glyceride.

Because moreover the degree of rearrangement Re is limited

5   to a maximum of 0.95 the process can be carried out with a high initial throughput in a packed bed reactor, using the short contact times known from WO96/14756 which makes the process cost effective.

In the first hour that the reaction is run the residence

10 time of the oil in the reactor is preferably <25 min, more preferably <20 min, still more preferably <15 min.

Residence time in hours is determined by dividing the volume of the catalyst bed by the volume of the oil passing the bed

15 in one hour.

In contrast to the 1500 kg oil throughput per hour per 400 kg catalyst in a packed bed reactor, which is recommended by the supplier of the above catalyst, the present invention allows the processing of 4400 kg oil per hour with the same

20 amount of enzyme. This corresponds with 32 minutes, and 11 minutes residence time respectively.

Initial throughput is based on fresh enzyme and obviously throughput is reduced to compensate for gradual loss of

25 enzyme activity.

Equally, when using a batch process, cost effectiveness may be enhanced by a considerable reduction of the catalyst concentration which in the present process may be lower than the 10 wt.% of the above mentioned prior art processes. The

30 catalyst concentration is selected from the range 0.05 - 9 wt.%, preferably 0.05 - 5 wt.%, more preferably 0.05 - 3 wt.% calculated on the reaction mixture.

Critical for the effect of the invention is that the
catalyst activity exceeds the specified minimum value being
250 IUN.

5 In contrast to what is expected from a low water and a low
diglyceride concentration, randomisation of the middle
position of the glyceride molecule proceeds to a
surprisingly high conversion degree in the present process.
The effect is that even before a 100% rearrangement degree
10 of the terminal positions has been attained, the degree of
middle position randomisation is substantial. This is
expressed in a high ratio Ra/Re which is expressed by the
equation Ra > 0.32Re − 0.08, preferably Ra > 0.32Re − 0.06
more preferably Ra > 0.32Re − 0.04, where Ra is the
15 conversion degree of the middle position randomisation and
Re is the conversion degree of overall rearrangement.

For acting properly the Lipozyme TL IM catalyst does not
need addition of water to the reaction mixture. The present
20 process is carried out with a reaction mixture in which the
content of water is low, preferably being in the range 0.001
− 0.1 wt.%, more preferably being in the range 0.001 − 0.05
wt.%. Measurement of the water content is done not earlier
than 30 minutes after mixing the catalyst with the feedstock
25 in a batch reactor. When a packed bed reactor is used,
measurement is done in a sample of the oil leaving the
reactor but not earlier than 30 minutes after startup of the
reactor.

30 Water content is determined by means of Karl Fischer
titration in a reaction sample from the reaction product
which was allowed to stabilise for 30 minutes.

In the art of enzymatic rearrangement the presence of silica
is reported to catalyze randomisation of the middle

5 position. However, when using a process according to the
prior art but using the highly active TL IM lipase according
to the present invention, it was after a decrease of the
concentration of silica containing catalyst that a
surprising increase of middle position randomisation was

10 noticed.

Determination of the degree of conversion for the reaction,
Re, is established as follows:

15 For any starting fat blend, the feedstock, and for an actual
reaction product the overall fatty acids composition, the
composition of fatty acids on the middle position and the
triglyceride composition is established using common
analysis methods comprising FAME-analysis, the GLC/carbon

20 number method and the HPLC/silver phase method as are
described in for example EP 78568, EP 652289, JAOCS, (1991),
68(5), 289-293 and Hammond E.W.J., Chromatography, 203, 397,
1981.

25 Determination of the degree of conversion Re for the
enzymatically catalyzed reaction is based on the change of
the carbon number profile. For a specific reaction product
and its starting material the carbon number profile is
determined by silverphase chromatography following the prior

30 art methods as described above. The carbon number is the
total number of carbon atoms in the three fatty acid
residues of a triglyceride molecule.

The carbon number profile of a 1,3-specific fully randomized
product is a theoretical value calculated from the measured
triacylglyceride profile of the starting mixture.
Experimental information necessary to perform this
5 calculation can be obtained by analyzing the profile of
fatty acid residues of the fat composition and by analysis
of the profile of fatty acid residues situated at the 2-
position of the triacylglyceride using the above mentioned
methods.

10

The molar fraction of any specific fatty acid residue (pA)
on either the sn-1 or the sn-3 position (which terminal
positions of the glycerol backbone are assumed to be
identical) is calculated according to the following formula:

15

$$pA_{sn1,3} = (3 * pA_{total} - 1 * pA_{sn2}) / 2$$

The indicees "total" and "Sn2" relate respectively to the
molar fraction of the fatty acid in the total fatty acid
20 residue mixture of the fat and the molar fraction on the
middle position of the glycerol backbone.

For all occurring fatty acid residues having a molar
fraction larger than 0.002 the value $pA_{sn1,3}$ is established.
25 The distribution of these molar fractions should be
normalized to 1.0.

Subsequently, the triacylglyceride profile for 100% 1,3-
selective randomness is calculated by simple statistics
30 known to the man skilled in the art which is based on the
concept that a triacylglyerol e.g. of the type ABB occurs in
a mole fraction p(ABB):

$$p(ABB) = 2 * pA_{sn1,3} * pB_{sn2} * pB_{sn1,3}$$

From the resulting triacylglycerol profile the carbon number

5 profile can easily be derived.

The degree of conversion is calculated according to the
following scheme:
For each carbon number in the range 30 - 60 the differences
10 are calculated between the mole fraction at the begin of the
reaction and at the 100% 1,3-selective randomisation state.
The sum of the absolute values of these differences defines
the 100% absolute change of carbon numbers which points to
the 100% conversion state.
15

For a specific sample taken from the proceeding reaction in
the same way for each carbon number between 30 to 60 the
differences between the fraction at the begin of the
reaction and the fraction at the actual state of the
20 reaction product are calculated. Again the sum of the
absolute value of these differences is taken. This number is
the actual absolute change of carbon numbers.

The degree of actual conversion Re = (actual absolute change
25 of carbon numbers) / (100% absolute change of carbon
numbers).

This equation applies for triglyceride products where the
above 100% absolute change of carbon numbers is at least
30 0.15. If not, the degree of conversion is determined in an
alternative way:

The mole fractions are established for each triacylglyceride
of the type H3, H2O and H2L, where H indicates fatty acid
residues of palmitic or stearic acid, O of oleic acid and L
of linoleic acid. H3 denotes a triacylglyceride containing 3

5 H type fatty acid residues and so forth for H2O and H2L. For
each of these triglycerides the absolute change between the
molar fraction at the begin of the reaction and at the 100%
1,3-selective random state is calculated. The sum of the
absolute values of these changes defines the 100% degree of

10 conversion, the 100% absolute change of triacylglyceride
groups.

For a specific sample of the proceeding reaction the
difference between the mole fraction at the begin of the

15 reaction and at the actual state of the reaction product is
calculated for the same triglycerides. The sum of the
absolute value of these changes is taken. This number is the
actual absolute change of triacylglyceride groups.

20 For the specific sample the degree of conversion Re follows
from the equation:

Re = (actual absolute change of triacylglyceride groups) /
(100% absolute change of triacylglyceride groups)

25

Determination of Ra, the degree of randomisation at the
middle position:

30 The degree of randomness Ra is calculated from the profile
of fatty acid residues of the total fat composition and the
profile of fatty acid residues situated at the 2-position of
the triacylglyceride. For establishing these data generally

GLC-FAME and 2-position analysis of the initial feedstock
and of the reaction mixture is used according to the methods
in the references described above.

5 For each fatty acid occurring in the triacylglyceride fat at
a molar level of at least 0.002 the absolute difference of
its Sn2 molar fraction at the begin of the reaction and its
molar fraction in the total triacylglyceride fat is
calculated. The sum of the absolute values of these changes
10 for all fatty acids defines the status of 100% degree of
randomness, denoted as Fa-Sn2-100%.

For a sample of the proceeding reaction for each of the same
fatty acids in the same way the absolute change between the
15 Sn2 molar fraction at the begin of the reaction and at the
actual state of the reaction product is calculated. Again
the sum of the absolute values of these changes is taken,
which number is the actual absolute change of fatty acids on
the Sn-2 position, denoted as Fa-Sn2-actual.
20

For the specific sample the degree of randomness Ra follows
from the equation: Ra = (Fa-Sn2-actual) / (Fa-Sn2-100%)

25 The temperature of the reaction mixture preferably is
selected from the ranges 40-85°C, preferably 45-80°C, more
preferably 50-75°C.

The process of the invention can be applied on all kinds of
30 triglyceride fat blends, but is most appropriate for
triglyceride fat blends in which there is an imbalance in
the distribution of fatty acids over the three glyceride
positions. Similarly to chemical interesterification the

present process is able to increase the content of triglycerides with a saturated middle position which is a much desired feature for improving fat qualities.

5 The process is particularly suitable for feedstocks containing
- any mixture of a liquid oil and a fully hydrogenated oil,
- any triglyceride fat which has not been subjected to hydrogenation, preferably
10 - a mixture of palm fat or a palm fat fraction and a lauric fat or a lauric fat fraction.

The invention also comprises the use of an aggregate of Thermomyces lanuginosa lipase and silica for partially
15 rearranging fatty acid residues of a triglyceride fat to a degree of 0.3-0.95, comprising a rearrangement on the middle position to a degree of 0.06-0.75, which lipase/silica aggregate has an activity of at least 250 IUN, preferably at least 300 IUN, more preferably at least 350 IUN.
20

An economy related benefit of the present process is that it does not need a cumbersome final purification from diglycerides as is necessary in the process of EP 652289.

25 The invention also comprises enzymatically rearranged triglyceride fats of which the degree of rearrangement Re is selected from the range 0.3-0.95 and of which the degree of glyceride middle position randomisation Ra is selected from the range 0.06-0.75, while Ra > 0.32Re - 0.08, preferably Ra
30 > 0.32Re - 0.06, more preferably Ra > 0.32Re - 0.04, where the parameters Re and Ra are as defined elsewhere in this specification.

Fats according to the invention are suitable for the preparation of food compositions, particularly for the preparation of a constituting fat phase which comprises a liquid oil and a structuring fat. Such fat phases are widely

5   used for the preparation of fat continuous emulsions used in the manufacture of e.g. spreads.

Because an enzymatic rearrangement process qualifies as natural, those fats too may be qualified as natural.

10 The present process allows the production of fats enriched with triglycerides which have a saturated fatty acid residue on the middle position. This is an important feature for subsequent processing of such fat in food applications where the crystallisation behaviour of the lipid phase is critical

15 for end product quality. Those triacylglycerides strongly influence both stability of the end products against adverse temperature conditions and crystallisation related process parameters based on the intrinsic crystallisation properties of the fat.

20 So the present process allows the production of the high palmitic fats which according to EP 831711 have a low graininess risk. This means that when the feedstock is a blend with palm oil or a palm oil fraction, the rearranged fat phase does not cause graininess in fat continuous

25 emulsion spreads prepared with such fat phase, or at least the graininess risk is substantially reduced.

The invention also comprises the food products in which a

30 fat is incorporated which is obtained by the process of the present invention.

The activity value of the used ER catalyst is measured as
follows:

5 **ESTABLISHING THE ACTIVITY OF THE LIPOZYME TL IM CATALYST**

1. PRINCIPLE

10 The method is provided by the catalyst supplier and is based
on interesterification of triglycerides by an immobilized
lipase. The conversion of tristearin in a substrate composed
of 27 w/w % fully hydrogenated soy bean oil and 73 w/w %
refined, bleached and deodorized soy bean oil at 70 °C and
15 200 rpm stirring is used to quantify the activity of the
catalyst. The concentration of tristearin is determined
using an HPLC based analytical method.

20
2. SPECIFICITY AND SENSITIVITY

Components having the same retention time as tristearin in
the chromatographic method used will cause an overestimation
25 of the tristearin concentration.

3. DEFINITION OF UNITS
30
The interesterification activity is defined as the initial
conversion rate for tristearin at standard conditions. 1
IUN corresponds to a conversion rate of 0.01 g tristearin/l
/minute/ gram catalyst.

35

4. APPARATUS

Analytical balance

Shaker water bath

Pipettes
Positive displacement pipette for taking oil samples (due to
high viscosity of the liquid).
5 Standard pipettes (e.g. Finnpipette) for the other steps in
the analysis.

HPLC   HPLC system including the following modules: HPLC
pump, injection system and column heater.
10
Light scattering detector, e.g. Sedex 55.

HPLC column   Column RP 18e (5µm), LiChroCart 250-4.

15 Data acquisition   Data can be acquired and processed using
chromatography software.

ANALYTICAL CONDITIONS
20
Column temp.:   40°C

Flow: 1.5 mL/min.

25 Mobile phase: The mobile phase consists of 50 v/v %
acetonitrile, HPLC grade and 50 v/v %
dichloromethane, HPLC grade.

Injection volume: 20 µL.
30
Run time: At least 11 minutes.

Column temperature: 40°C (±2°C)

35 Detector: 40°C (±2°C), nitrogen pressure 2.3 bar.

Detector sensitivity: Gain 6

40
5. REAGENTS AND SUBSTRATES

CHEMICALS

45 Tristearin. SIGMA grade, Approx. 99%

SOLUTIONS

Substrate (20 grams):

27 w/w % fully hydrogenated soy bean oil (delivered by ADM, Illinois, USA)

5 73 w/w % refined, bleached and deodorized soy bean oil (delivered by ADM, Illinois, USA)

Catalyst (2 gram): The samples are conditioned at a_w =0.3 for at least 24 hours.

10

6. SAMPLES AND STANDARDS

15 STANDARDS

A standard curve of tristearin is made in the concentration range from 0.25 to 2.0 mg/ml.

LEVEL CONTROL

20 Reference sample: Lipozyme TL IM reference PPW6503-3, particle size fraction 425-500µm, is used (single determination).

7. PROCEDURE

25

As substrate a blend of 27 w/w % fully hydrogenated soy bean oil (FH SBO) and 73 w/w % refined, bleached and deodorized soy bean oil (RBD SBO) is made. The oil mixture is heated to 80 °C in a water bath, and is well mixed.

30

The oil mixture is weighed out in 100 ml conical flask with a screw top wail, about 20 grams in each. The precise weight is notated with 2 decimals. The flasks (batches) are placed in the shaker water bath at 70°C and 200 rpm.

A catalyst amount corresponding to approx. 10% of the
amount of oil is weighted out (approx. 2 grams). The
precise weight of the catalyst is determined with 2
5 decimals.

When the oil mixture is homogeneous, a time zero-sample is
taken. 100µl of the oil is taken with a positive
displacement pipette (Gilson microman). The pipette tip is
10 wiped off by a Kleenex to remove outside oil mixture and the
sample is deposited in a HPLC vial (type BROWN 12x32mm with
Silicone/PTFE Septa).

The weighed amount of catalyst is added to the flasks
15 containing the oil mixture and samples are taken to the
times: 15, 30, 45 and 60 minutes. All samples are 100 µl.
The samples are stored in a freezer until HPLC analysis.

The samples in the HPLC vials are diluted with 900 µl
20 dichloromethane. This solution is mixed on a whirl-mixer,
before a further dilution of 100 µl to 900 µl
dichloromethane is made (total dilution of 100x). After a
further mixing this sample is being analysed by HPLC.

25 The diluted samples are analysed by HPLC-ELSD.

The response versus concentration of the tristearin
standards are fitted to an exponential model.

30
8. CALCULATION

18

The concentration of tristearin in the samples is calculated by use of the standard curve.

The conversion of (the decrease in) tristearin concentration
5 versus time is fitted to a exponential model, by non linear parameter estimation. The model is:

$$C_{Tristearin}(t) = C_{0,est} \cdot \exp(-k_{est} \cdot t)$$

10

Where

$C_{tristearin}(t)$ = is the concentration of tristearin in the reaction mixture at time, t.

15 $C_{0,est}$ = start concentration of tristearin (estimated parameter)

k_{est} = rate constant (estimated parameter)

t = reaction time

20 From the estimated rate constant, k_{est}, the activity at standard conditions, the IUN activity, can be calculated according to the following formula:

$$IUN/g = k_{est} \cdot C_{0,std} \cdot 100 \cdot \frac{W_{std}}{W} \frac{M_{oil}}{M_{oil,std}}$$

25

$C_{0,std}$ = start concentration of tristearin at standard condition.

W_{std} = weight of catalyst under standard conditions (2g)

W = actual weight of catalyst

30 M_{oil} = actual weight of oil

$M_{oil,std}$ = weight of oil under standard conditions (20g)

Example 1

60 g of palm oil and 40 g of palm kernel oil were mixed together in a 100 ml reaction vessel and heated to 70°C. 1 wt.% of Lipozyme TL IM (activity 455 IUN) was added to the mixture and this was stirred at 70°C for 10 hours. Samples were taken at intervals and the degree of conversion Re and the degree of randomisation Ra were determined according to the methods described elsewhere in this specification by means of establishing changes in carbon number distribution and changes in composition of fatty acids on the middle position.

At the conversion degrees Re of 0.85 and 0.5, the following degrees of randomisation of the middle position have been found:

TABLE I

Degree of Conversion Re	Degree of randomisation Ra
0.85	0.28
0.5	0.1

Example 2

Example 1 has been repeated but using a low acticvity TL IM lipase catalyst and the 10% catalyst concentration as used in the prior art.

Example 3

Example 1 has been repeated using the same high activity TL IM catalyst, but with the same high catalyst concentration as used in comparison example 2, allowing a contact time which is much shorter than necessary in examples 1 and 2.

TABLE II

ER using TL IM catalyst	Example 1	Example 2	Example 3
Catalyst conc. (wt.%)	1	10	10
Water (wt.%)	0.013	0.025	0.024
Reaction time (h)	5.25	2.75	0.60
Activity (IUN)	455	160	455
Conversion Re	0.85	0.85	0.85
Randomness Ra	0.28	0.08	0.21

5 The high activity TL IM catalyst (example 1) causes at a low
concentration the same conversion Re as the prior art low
activity catalyst of example 2 but accompagnied by a high
randomisation of the middle composition. When increasing the
catalyst concentration according to example 3 the same
10 effect as example 1 is obtained with a shorter contact time,
which enables a higher throughput.

Examples 4 - 6

15 Examples 1 - 3 have been repeated under the same conditions,
respectively, but using shorter contact times. The shorter
contact times result in a lower degree of conversion, but
even at these lower conversion degrees the same relatively
high randomisation on the middle position is observed.
20

TABLE III

	Example 4	Example 5	Example 6
Conversion Re	0.4	0.4	0.4
Randomness Ra	0.08	0.02	0.08

CLAIMS

1. Process comprising the exposure of a triglyceride fat to a catalyst comprising a lipase which process results into a rearrangement of the fatty acid residues on the glyceride moiety over the terminal and middle positions, which rearrangement proceeds to a degree of conversion Re, where Re is selected from the range 0.3-0.95, the rearrangement comprising a randomisation of the fatty acid residues on the middle position to a degree of conversion Ra, where Ra is selected from the range 0.06-0.75, which process is characterized by the use of a Thermomyces lanuginosa lipase containing catalyst having an activity which is at least 250 IUN, while Ra is greater than 0.32Re - 0.08, the catalyst activity and Ra and Re being as defined in the specification.

2. Process according to claim 1, characterised in that the catalyst has an activity of at least 300 IUN, more preferably at least 350 IUN.

3. Process according to claims 1 or 2, characterised in that Ra is greater than 0.32Re - 0.06, preferably greater than 0.32Re - 0.04.

22

4. Process according to anyone of claims 1 - 3, characterised in that

the amount of catalyst when used in a batch reactor is 0.05 - 9 wt.%, preferably 0.05 - 5 wt.%, more preferably 0.05 - 3 wt.% calculated on the reaction mixture.

5. Process according to anyone of claims 1 - 4, characterised in that in the first hour that the reaction is run in a packed bed reactor the residence time of the oil in the reactor is <25 min, preferably <20 min, more preferably <15 min.

6. Process according to anyone of claims 1 - 5, characterised in that the triglyceride fat is selected from the list consisting of

- any mixture of a liquid oil and a fully hydrogenated oil,
- any triglyceride fat which has not been subjected to hydrogenation, preferably
- a mixture of palm fat or a palm fat fraction and a lauric fat or a lauric fat fraction.

7. Process according to anyone of claims 1 - 6, characterised in that the rearrangement degree Re is less than 0.9, preferably less than 0.85.

8. Process according to anyone of claims 1 - 7, characterised
   in that the rearrangement degree Re is at least 0.35,
   preferably at least 0.4.

9. Process according to anyone of claims 1 - 8, characterised
   in that the content of water in the reaction product is
   selected from the range 0.001 - 0.1 wt.%, preferably from
   the range 0.001 - 0.05 wt.%.

10. Process according anyone of claims 1 - 9, characterised in
    that the temperature of the reaction mixture is selected
    from the range 40-85°C, preferably from the range 45-80°C,
    more preferably from the range 50-75°C.

11. An enzymatically rearranged triglyceride fat of which the
    degree of rearrangement Re is selected from the range 0.3-
    0.95 and of which the degree of glyceride middle position
    randomisation Ra is selected from the range 0.06-0.75, while
    Ra is greater than 0.32Re - 0.08, preferably greater than
    0.32Re - 0.06, more preferably greater than 0.32Re - 0.04,
    where the parameters Re and Ra are as defined in the
    specification.

24

12. A triglyceride fat according to the preceding claim which is obtained by enzymatic rearrangement of a fat selected from the list consisting of

- any mixture of a liquid oil and a fully hydrogenated oil,
- any triglyceride fat which has not been subjected to hydrogenation, preferably
- a mixture of palm fat or a palm fat fraction and a lauric fat or a lauric fat fraction.

13. A triglyceride fat according to claim 11 or 12, characterised in that the rearrangement degree Re is less than 0.9, preferably less than 0.85.

14. A triglyceride fat according to anyone of claims 11 - 13, characterised in that the rearrangement degree Re is at least 0.35, preferably at least 0.4.

15. Use of an aggregate of Thermomyces lanuginosa lipase and silica for partially rearranging fatty acid residues of a triglyceride fat to a degree of 0.3-0.95, comprising a rearrangement on the middle position to a degree of 0.06-0.75, which lipase/silica aggregate has an activity of at least 250 IUN, preferably at least 300 IUN, more preferably at least 350 IUN.

16. A food products which comprises a fat according to anyone
of claims 11 - 14.

26

EPO - DG 1
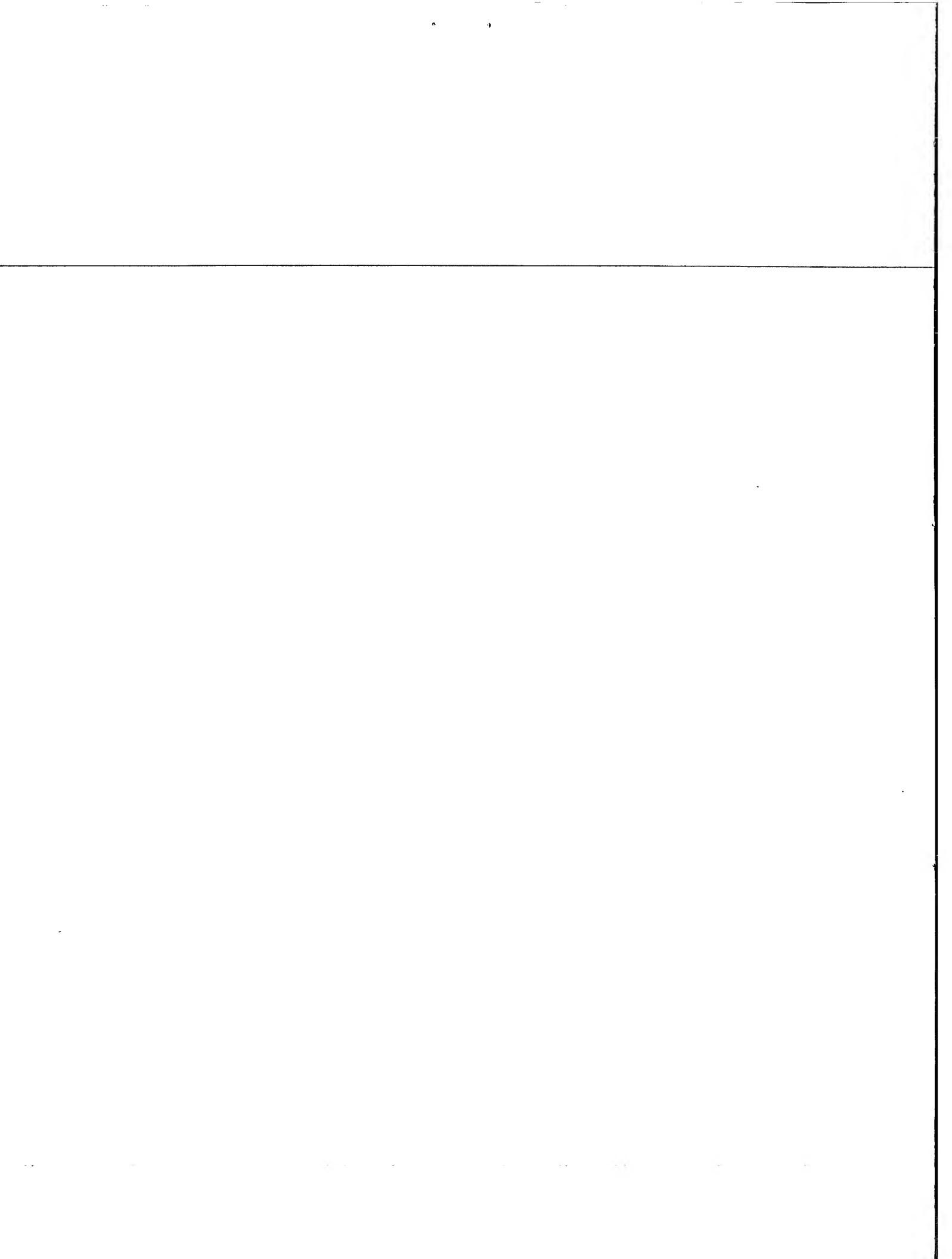
2 6. 01. 2004

(102)

ABSTRACT

Process comprising the exposure of a triglyceride fat to a
catalyst comprising a lipase which process results into a
rearrangement of the fatty acid residues on the glyceride
moiety over the terminal and middle positions, which
rearrangement proceeds to a degree of conversion Re, where Re
is selected from the range 0.3-0.95, the rearrangement
comprising a randomisation of the fatty acid residues on the
middle position to a degree of conversion Ra, where Ra is
selected from the range 0.06-0.75, which process is
characterized by the use of a Thermomyces lanuginosa lipase
containing catalyst having an activity which is at least 250
IUN, while Ra is greater than 0.32Re - 0.08.